

GROWTH STUDIES ON CILIATES

VI. DIAGNOSIS, STERILIZATION AND GROWTH CHARACTERISTICS OF *PERISPIRA OVUM*

VIRGINIA C. DEWEY¹ AND G. W. KIDDER

(*Arnold Biological Laboratory, Brown University and the Marine Biological
Laboratory, Woods Hole, Mass.*)

The growth characteristics of a number of species of ciliates have been reported recently in the literature. Most of these papers deal with saprozoic forms (Phelps, 1935, 1936, etc.) or with bacteria-feeders (Johnson, 1933; Kidder and Stuart, 1939*b*). The report which is to follow deals with observations of a quantitative nature made on the holotrichous ciliate, *Perispira ovum*, and represents the first attempt at an analysis of the growth characteristics of an obligate carnivore in bacteria-free medium, although Brown (1940) has published a brief account of some of the growth characteristics of *Leucophrys patula*, a facultative carnivore. Due to the fact that *Perispira* is not a common genus and its previous descriptions have been contradictory (Stein, 1859; Levander, 1894; Kahl, 1926), a rather complete description of its organization will be given. This description will serve to establish the identity of our experimental organism and should eliminate confusion in future investigations, regarding its specific designation. We agree with the views expressed by Taylor and Furgason (1938) and Furgason (1940) that the space devoted to a purely morphological description is warranted as a means of standardization of experimental material.

MATERIAL AND METHODS

During the summer of 1939 a number of specimens of *Perispira ovum* were found in a sample taken from a fresh water stream on Gifford St. in the town of Falmouth, Massachusetts. This sample contained many *Euglena* along with numerous other species of protozoa. The *Perispira* were isolated and maintained in mass culture, along with their associated bacteria and *Euglena gracilis*, for a period of two months before any attempt was made at sterilization. During this period care had to be taken to make frequent sub-cultures, as heavy bacterial growth proved to be detrimental to the *Perispira*.

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Description of Perispira ovum

The ciliate is a member of the family Spathidiidae. It is evenly oval in shape, its size varying with the state of its nutrition. Well-fed organisms measure $65\ \mu$ – $120\ \mu$ in length \times $50\ \mu$ – $110\ \mu$ in width, while starved ciliates are much smaller ($30\ \mu$ – $60\ \mu \times 20\ \mu$ – $45\ \mu$). The most characteristic structure of the body is a spiral ridge, originating at the dorsal anterior portion of the body. From the point of origin the ridge bends sharply to the right and then spirals posteriorly toward the left, ending on the left side of the body near the posterior end (Fig. 1).

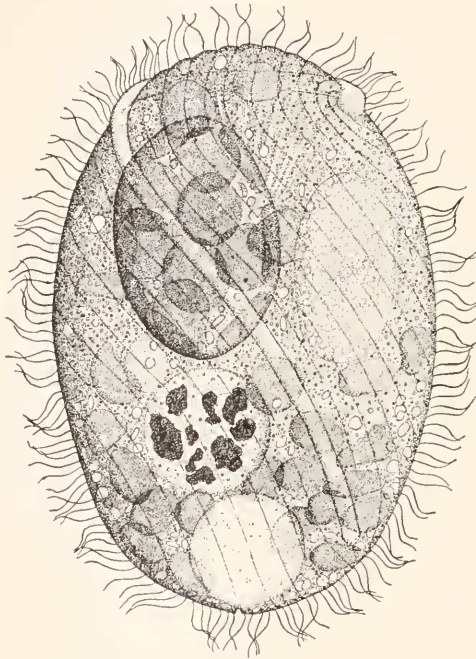


FIG. 1. Living ciliate, left side $\times 1000$. The slender refractile trichites may be seen extending from the anterior end into the endoplasm. To the right of the drawing is the macronucleus; toward the posterior end are the contractile and excretory vacuoles, the excretory pore and the scattered chloroplasts and paramylum bodies. A single *Euglena* has recently been ingested.

Thus the ridge describes only one complete turn about the body, whereas Kahl (1926) figures an extra half turn. This ridge is composed of structureless, homogeneous protoplasm throughout most of its length, but in the anterior third it bears a narrow groove. This groove is slightly expanded very near its beginning into what appears to be a

small pore (Fig. 2). The pore and the groove represent the mouth, which is open only during the few seconds of food ingestion. The mouth can be seen in the non-feeding organism only after appropriate preparation (opal blue or nigrosin). Extending from the mouth and running into the endoplasm are a number of refractile and delicate trichites (Fig. 1). In most living specimens a few of these trichites may be seen lying loose in the endoplasm, but the majority are arranged as are those in a typical *Spathidium*. We have been unable to demonstrate them with any of the standard techniques. Levander (1894)

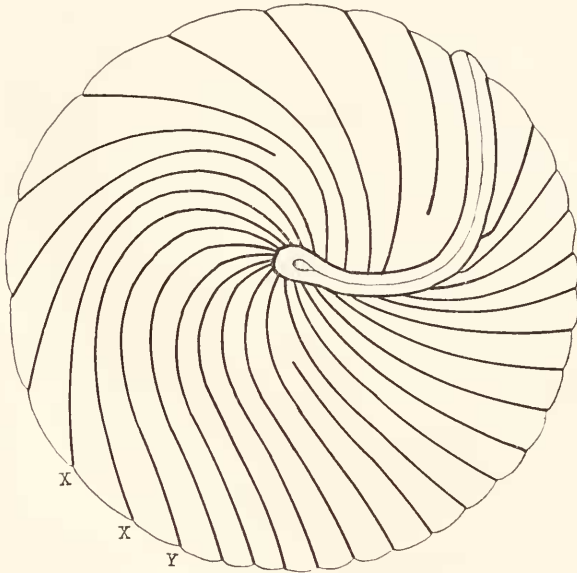


FIG. 2. Diagrammatic representation of the anterior pole, showing the ridge and the origins of the ciliary lines. The mouth is indicated in the ridge. *x, x* represent the ciliary lines which bear the paired, short bristles. *Y* represents the ciliary line which bears the long, unpaired bristles.

saw these trichites and included them in his figure but interpreted them as forming a basket. Kahl (1926) figures the trichites but refers to them as "Trichocysten" and describes them as being present in the protoplasm of the ridge. We have been unable to verify this last observation and there is no evidence from a study of the activity of a feeding animal that it possesses any trichocysts which function as do those in *Spathidium*.

The disposition of the cilia may be observed clearly in small organisms which are devoid of food. Relief staining methods with opal blue or nigrosin on medium-sized or small ciliates give excellent and

striking results and permit accurate analysis of the relative size and distribution of the motor organelles (Fig. 3, *A* and *B*).

On either side of the spiral ridge there is a row of closely set cilia. These rows join at the anterior end of the ridge but remain separate at its posterior end. This arrangement makes, in effect, a continuous row of cilia starting at the posterior end of the ridge, encircling the anterior end, and returning on the opposite side of the ridge. Contrary to the statement of Kahl (1926), we have found these cilia to be identical in size but much more closely set than the rest of the peripheral

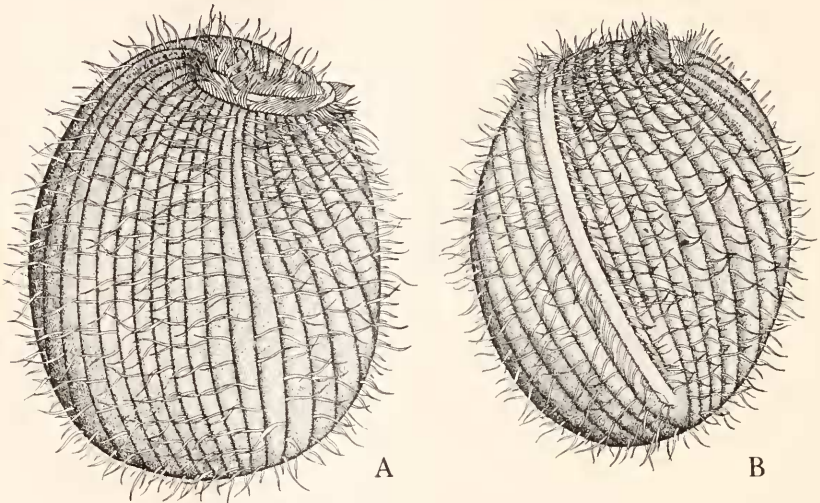


FIG. 3. Drawings of opal blue preparations of medium-sized organisms to show the size and arrangement of the cilia and the disposition of the ciliary rows. Organisms slightly flattened in preparation. $\times 700$. *A*. Right side. Note the three rows, bearing bristles, toward the left of the drawing. *B*. Left side, showing the ridge.

cilia. It seems probable that these closely set cilia were mistaken by Kahl for the trichocysts which he describes as being present in the ridge (Fig. 3, *A*).

The general peripheral ciliary rows originate from the edges of the ridge in the region of the anterior pole. These rows spiral around the body from right to left, paralleling the ridge. They end irregularly in the region of the posterior pole. There are 26 complete rows of fairly widely placed cilia, with from one to three interpolated rows. The general arrangement of these rows may best be understood by referring to Fig. 2. Three of these peripheral rows deserve special attention, since they are made up of diverse types of structures. Two of the rows,

originating from the anterior tip of the ridge, bear short, paired bristles (Fig. 2, *x, x*; Fig. 3, *A* and *B*). These bristles are distributed along the first quarter of the length of the row and appear to originate from basal bodies directly in the row. They may be observed in living organisms and are clearly demonstrated after relief staining. They appear to vibrate rapidly but through extremely short arcs. They correspond to the "Chemoreceptoren" described by Gelei (1933) in *Trachelophyllum*, except that he maintains that their origin is between the ciliary rows. We cannot subscribe, at present, to Gelei's theory as to their function. There is no evidence that *Perispira* receives chemical stimulation through these bristles. Just to the right of the rows which bear the short bristles is a single row of long, stiff bristles (Fig. 2, *y*; Fig. 3, *A* and *B*). These are unpaired and occupy most, but not all, of the length of the row, which is completed posteriorly by normal flexible cilia. These long bristles correspond to the "Tastborsten" of Gelei (1933), but do not appear to function as tactile receptors, as he supposed they did in *Trachelophyllum*. Contact with prey in the region of these long bristles evokes no apparent response in *Perispira*. Another region where touch stimuli are received will be described shortly when the mechanism of feeding is considered.

The endoplasm of *Perispira* is coarsely granular and contains many different types of inclusions. Well-fed organisms are bright green due to the tightly packed chloroplasts of the ingested *Euglena*. The protoplasm of the *Euglena* is digested first, releasing the chloroplasts and the paramylum bodies. Next the chloroplasts are broken down and the unassimilated material deposited in a vacuole, as a reddish-brown mass, for defecation. Animals in the early stages of starvation contain quantities of paramylum bodies, which gradually disappear, leaving the *Perispira* relatively clear. These paramylum bodies appear to function as reserve food, enabling the ciliate to continue living long after all the *Euglena* have been ingested.

A single contractile vacuole is located at the posterior end of the body. It empties its contents to the outside through a permanent pore, which is always visible at the extreme posterior pole (Fig. 1).

The macronucleus is quite large and coarsely granular. It is variable in shape, being ovoid to elongate or even horseshoe-shaped. In starved animals it regularly breaks up into two spheres, as described by Levander (1894). After staining with haematoxylin, two types of granules are demonstrated, the fine chromatin granules and larger spheres of deeply-staining substances lying in vacuoles. Their appearance corresponds to that of the spheres found in such forms as *Paramecium*

(Wenrich, 1926) and *Diophrys* (Summers, 1935). After the Feulgen reaction, however, only the chromatin granules are stained and the vacuoles which hold the larger spheres appear hollow, giving the macronucleus an areolar appearance.

There is a single micronucleus in *Perispira ovum* which is very low

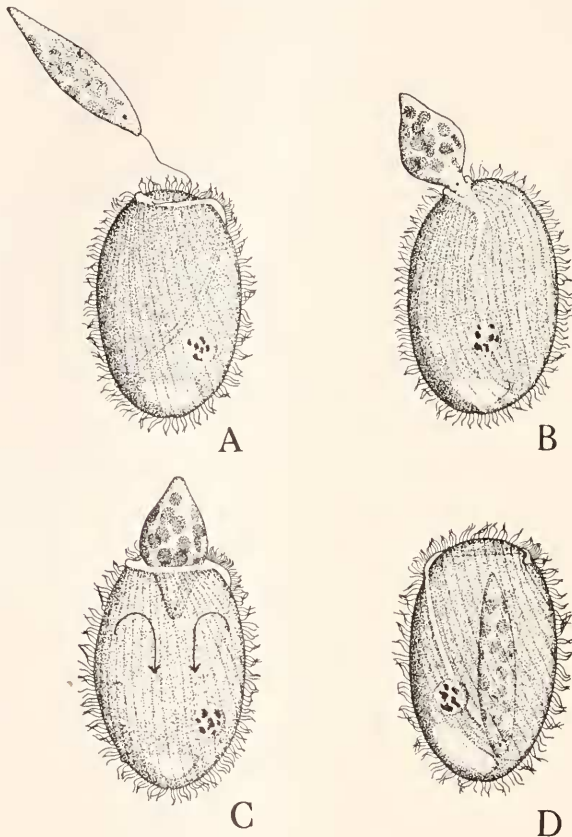


FIG. 4. Starved *Perispira ovum*, showing successive stages during the ingestion of a *Euglena*. A. Flagellum is caught. B. Mouth beginning to open. C. Arrows indicate the direction of the cyclosis which carries the *Euglena* in. D. Mouth closed immediately after ingestion.

in chromaticity. It regularly lies very close to the macronucleus and may easily be overlooked. Kahl (1926) states that he was never able to identify it with certainty.

We have made extensive observations on the mechanism of feeding. Food taking is best observed by placing a drop of a culture containing starved *Perispira* on a slide and adding to this drop a number of active

Euglena. Within a few seconds almost all of the ciliates will have started feeding and the whole process can be studied under an oil immersion lens.

The ciliates swim with a rotating motion which is uninterrupted by contact with other ciliates or with rounded *Euglena*. When the flagellum of an active *Euglena* comes in contact with any part of the ridge, however, the motion of the *Perispira* changes and it stops its rotation. If the contact has been made near the anterior end of the ridge (Fig. 4, *A*), the flagellum is rapidly drawn in, dragging the flagellate up to the mouth. The ridge immediately opens along the seam (Fig. 4, *B*) and the *Euglena* is drawn into the body of the *Perispira* (Fig. 4, *C* and *D*). The mouth then closes and the whole process may be repeated many times in the course of a few minutes. The force which draws the prey into the mouth is manifested by the increased intensity of the cyclosis of the protoplasm of the ciliate, which creates a current directed inward from the mouth opening (Fig. 4, *C*). Ingested *Euglena* are not surrounded by a distinct vacuole, but appear to lie free in the endoplasm of the ciliate. Killing of the prey is very slow and it is not uncommon to see four or five ingested flagellates contracting and expanding within the body of the *Perispira*.

Although we placed *Perispira* in fluid containing numerous species of protozoa, we have never observed it ingesting any types except euglenoid flagellates. It may be that its specialization has gone so far that its prey must possess certain structural characteristics in order to evoke the feeding response.

Sterilization and Establishment in Bacteria-free Culture

Advantage was taken of the fact that well-fed *Perispira* are positively geotropic. One to two milliliters of a heavy suspension was placed in the top of a special tube filled with sterile water (pH 7.0). The tube was constructed with alternating shelves in order to prevent any clumps of débris from falling to the bottom. After the majority of the ciliates had migrated around the shelves to the bottom of the tube they were drawn off into a sterile, cotton-plugged centrifuge tube. The ciliates were then centrifuged slowly until concentrated, the supernatant fluid withdrawn with a sterile serological pipette and more sterile fluid added. This washing process was repeated ten times, after which the ciliates were placed in the top of a 50 ml. burette full of sterile water. After this second migration had been completed and a large number of the ciliates withdrawn from the bottom of the burette into a sterile cotton-plugged tube, they were placed in a Syracuse watch glass

enclosed in cellophane (Kidder, Lilly and Claff, 1940). Single ciliates were then withdrawn by means of sterile capillary pipettes and placed into other similar dishes, from which they were then removed to tubes containing either water or 0.5 per cent Yeast Harris.

The above method yielded several sterile ciliates. The various steps described were necessary, inasmuch as this ciliate possesses rather uneven contours and is not easily washed free of its adhering bacteria. All cultures finally labeled as sterile were so designated only after the various sterility tests had been used in accordance with our previously described methods (Kidder and Stuart, 1939a).

The washed ciliates were placed into: (1) water (pH 7.0) or (2) 0.5 per cent filtered Yeast Harris. To both of these fluids sterile *Euglena* had previously been added. The method of sterilization of our strain of *Euglena* has been reported elsewhere (Kidder, 1940). Growth of the ciliates was rapid in some tubes but extremely slow in others. It soon became evident that the tubes in which rapid multiplication occurred were bacterially contaminated, while the others were sterile. We considered the possibility that the bacteria might be furnishing some substance needed for normal growth. Accordingly a suspension of *Phytomonas* (the contaminating bacterium from one of the growing cultures) from an agar slant was made in water (pH 7.0) and autoclaved for 20 minutes at 120° C.; 0.5 ml. of this suspension was added to a tube containing sterile ciliates in water with *Euglena*. A Seitz filtrate of a 24 hr. culture of *Phytomonas* in 0.5 per cent Yeast Harris was also prepared and 0.5 ml. added to another tube containing sterile ciliates in water with *Euglena*. In both tubes the ciliates immediately began to multiply and proved to be sterile.

Loop transplants were made from the first of these cultures into: (1) autoclaved *Aerobacter* in casein peptone (Peptone Roche) and (2) autoclaved *Phytomonas* in Yeast Harris to both of which *Euglena* had been added. Growth was good in both media, but in all cases appeared slightly better without Yeast Harris. Transplants from these cultures into Difco Tryptone and *Euglena*, without autoclaved bacteria, proved successful and the cultures have been carried in 1.0 per cent Tryptone since then.

After nearly a year of continued culture in Tryptone and *Euglena*, we feel sure that the function of the autoclaved bacteria and of the bacterial filtrate in our initial establishment experiments was simply to reduce the speed of the growth of the *Euglena* so that the single *Perispira* which were inoculated were not "overgrown." Later serial stock transplants were always made with many *Perispira* and the rapidly growing *Euglena* were quickly reduced in number.

Technique of Experimental Culture

All experimental cultures were grown in the special culture flasks described elsewhere (Kidder, 1941). These flasks are provided with a port plugged with a vaccine tip, which allows one to draw off samples for counting with little danger of contaminating the cultures. The volume of all cultures was 100 ml. Incubation was at $28^{\circ} \pm 1^{\circ}$ C. The cultures were grown in a box provided with a large water filter behind which a constant source of light was provided by means of a 50-watt electric bulb.

The fluid to be used in any set of experiments was autoclaved, allowed to cool and inoculated with *Euglena gracilis* and placed in the light box for from 24 to 48 hours. Just before the *Perispira* were inoculated the concentration of *Euglena* was determined by drawing out a sample and making cell counts with a Sedgwick-Rafter counting chamber and a Whipple micrometer. From a culture of *Perispira* two to three weeks old, the average size of the individuals having been determined previously, inoculations were made through the side arm of the flasks. The initial inoculum was determined for each flask by immediately withdrawing a sample and counting the number of *Perispira* in a unit volume. These counts were made with the aid of a dissecting binocular microscope, using the same system as has been reported for *Tetrahymena* (Kidder, 1941). Thereafter samples were taken at regular intervals and the concentrations of *Euglena* and *Perispira* determined. The average individual volume of the *Perispira* was calculated from measurements of 50 cells. Before measuring, the cells were fixed in the fumes of osmic acid. The volume was calculated by considering the individual cell as a prolate spheroid and applying the formula $V = 4/3 \pi ab^2$, where $2a$ = the major axis (length) and $2b$ = the minor axis (width).

GROWTH

Normal Population Curve

The growth of a population of *Perispira*, when they are inoculated into a flask of 1 per cent Difco Tryptone containing a concentration of *Euglena gracilis* of approximately 10,000 per ml., follows a reproducible and characteristic course. The shape of the curve depends on a number of conditions, one of which is the age of the inoculum. If *Perispira* reproducing at their maximum rate (logarithmic growth phase) are used as the inoculum, they continue to reproduce at this rate in the experimental flask. When the logarithms of the population densities are plotted against time the result is a straight line. During this

TABLE I

Average data from five experiments. Medium, 1 per cent Difco Tryptone; age of inoculum of *Perispira* 15 days; *Euglena* grown in the medium 34 hours before the *Perispira* were inoculated.

	Time in Hours										
	0	18	30	42	60	90	114	139	162	186	204
<i>Perispira</i> per ml.	100	93	145	235	554	4250	22920	57800	78100	79160	77700
<i>Euglena</i> per ml.	14640	70820	159600	282800	393400	289800	48240	2096	—	—	—
Indiv. Vol. in μ^3	21490	110750	147000	174400	206400	237400	126400	54750	36750	32440	31250

TABLE II

Medium—1 per cent Difco Tryptone; age of inoculum of *Perispira*—21 days; *Euglena* grown in the medium 48 hours before the *Perispira* were inoculated.

	Time in Hours												
	0	23.5	49.5	73	92.5	115.5	145	168	192.5	216.5	403	571	1003
<i>Perispira</i> per ml.	90	75	490	2000	10000	36500	85500	93500	112000	85000	62000	11750	350
<i>Euglena</i> per ml.	9500	53000	130000	255000	241000	11500	—	—	—	—	—	—	—

logarithmic growth phase the generation time (calculated from the formula $g = \frac{t \log 2}{\log b - \log a}$ where t = the time in hours during which the population has been increasing, a = the number of cells per unit volume at the beginning and b = the number of cells at the end of the time, t) is $10.5 \pm .3$ hours. When ciliates taken from the stationary phase (10 to 20 days old) are inoculated there follows a period of approximately

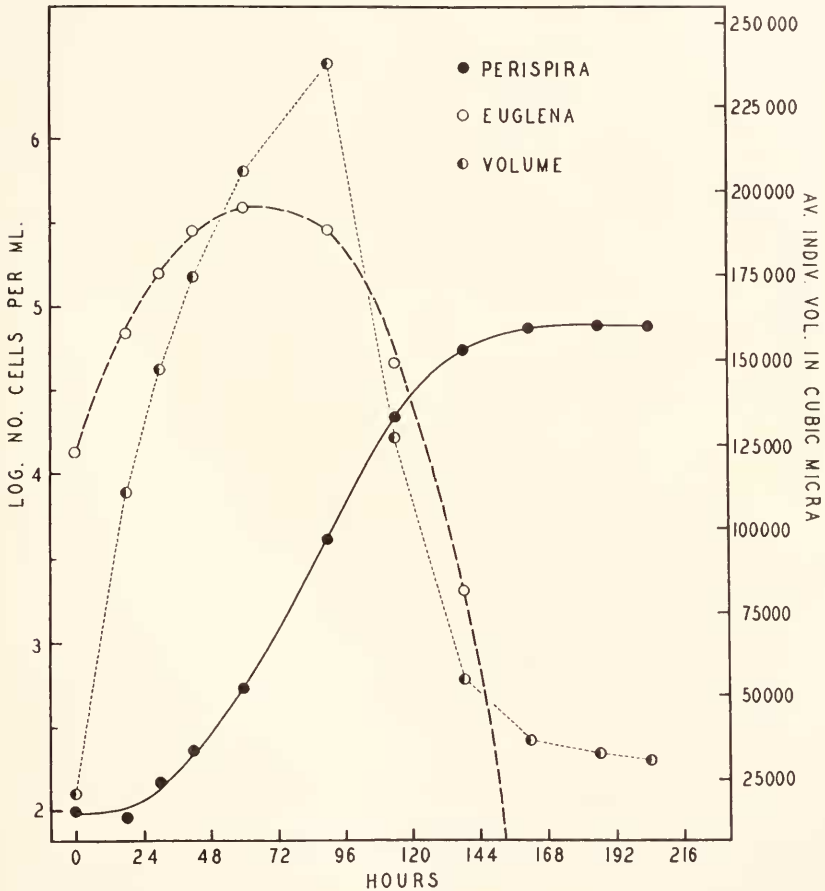


FIG. 5. Graphic representation of data presented in Table I.

24 hours during which there is no increase in the population. Usually some of the inoculated ciliates die during this lag phase. After a considerable number of *Euglena* have been eaten, reproduction follows and the culture goes into the logarithmic phase. The slope of the curve (and therefore the generation time) is identical with that for populations started with logarithmic ciliates (Table I; Fig. 5).

During the early phases of a culture the *Euglena* show a marked increase in concentration but the *Euglena* curve turns over rapidly as the *Perispira* increase. The *Euglena* curve represents the resultant of their own multiplication and their destruction by the *Perispira*. At about the 120th hour most of the trophic flagellates have been ingested (Fig. 5). These curves correspond, in general, to Fig. 6 in Brown's (1940) paper.

After most of the available flagellates have been eaten the *Perispira* continue to reproduce for some time at the expense of their individual size. Eventually the concentration reaches a relatively constant level at about 100,000 cells per ml. From then on the population declines slowly and there are still many small, trophic forms, capable of starting healthy cultures, even after two months. Table II gives the results of

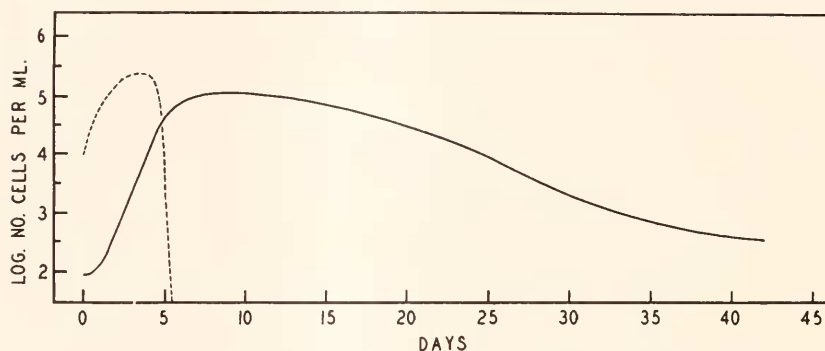


FIG. 6. Graphic representation of data presented in Table II.

an experiment carried for 42 days and these results are shown graphically in Fig. 6. Separate experiments, wherein large, medium-sized and small ciliates were selected for single cell inoculations, indicate that there is a relationship between the size of these starved cells and their ability to resume feeding and reproduction. This correlates with the deaths which occur upon mass inoculations into the experimental flasks. We believe that the variability in size during the late phases of a culture is due to purely fortuitous circumstances, however, and that the larger forms are those which were able to procure the last of the *Euglena*. Further investigations upon this point are needed.

Size Changes in Relation to Age of Culture

Ciliates taken from cultures three weeks old are very small and transparent. As was stated above, when these organisms are used as the inoculum a lag occurs before reproduction starts. It is during the

lag phase that the size of the individuals is increasing most rapidly. Feeding starts as soon as the *Perispira* and *Euglena* are brought together. The ciliates do not have to reach their maximum size, however, before they undergo reproduction. The individual size steadily increases during the logarithmic phase until the time when the food organisms begin to decrease in concentration. After this peak the size diminishes rapidly. The most rapid decrease in cell size corresponds to the upper limits of the logarithmic phase and the phase of negative growth acceleration. During the stationary phase the cell size is still decreasing, but very slowly (Fig. 5). This last and gradual decrease seems to be the result of the utilization of stored food materials while the previous rapid decrease was brought about largely by partition of protoplasm through rapid division.

The size changes hold for all types of media investigated so far and for the two species of euglenoid flagellates employed (*Euglena gracilis* and *Astasia klebsii*) as food organisms. Differences in speed of increase and maximum size attained were noted but these differences were slight.

Effect of Media, Light and Species of Food Organism

Having established the fact that the growth characteristics of *Perispira* in Difco Tryptone and *Euglena gracilis* were quite regular and reproducible, experiments were set up to determine what effects would be produced by varying the standard combination. Flasks were prepared as before except that 1 per cent Difco Proteose-peptone or 0.5 per cent Yeast Harris was substituted for the Tryptone. In other flasks sterile *Astasia klebsii* was substituted for *Euglena*; still other flasks were prepared with colorless *Euglena* (grown in the absence of light) in Tryptone. All the flasks were inoculated with *Perispira*, the first three types were incubated in the light box while the last was incubated in a constant temperature box (27° C.) in the dark.

The differences observed between the various types of experiments may be summarized briefly. In the colorless *Euglena* there were a few more deaths among the individuals in the initial inoculum than in the controls. The generation times in the logarithmic phase were identical, however, and the maximum concentration and longevity of the cultures were not significantly different. In Proteose-peptone the general shape of the curve was similar to that of the control, but the generation time was 12.2 hours as compared to 10.6 hours for the control. The Proteose-peptone cultures declined more rapidly than did the controls. When the *Perispira* fed on *Astasia* the generation time was greater than

in the controls (12.5 hours), the maximum concentration was much lower (13,000 as compared with 93,000 per ml.) and the decline was very rapid. Moreover the *Astasia* were never completely removed from the culture, as shown by the fact that after the *Perispira* had become reduced to 40 per ml. (17 days) the *Astasia* had multiplied so that the concentration was again appreciable. These declining ciliates refused again to feed on the *Astasia*. In Yeast Harris the ciliates inoculated had nearly all died in 24 hours and in 48 hours all were dead.

General Observations

There are a number of comments to be made which do not have a place in the experiments described above.

When *Perispira* was first isolated and was growing in association with bacteria many monsters invariably appeared in the heavy cultures. These monsters were sometimes two to three times the size of the largest normally shaped individuals and were quite irregular in outline. They appeared to be the result of a failure to divide, on the part of certain individuals, while the power to ingest food was retained. After the establishment of our cultures in bacteria-free media only occasionally were monsters observed. When these organisms were encountered they were not used either in the counts or in the measurements. Attempts to start cultures from these monsters always failed.

Although there is no evidence that *Perispira* forms cysts, conjugates or undergoes endomixis, a peculiar type of reaction is characteristic of organisms in the later stages of a culture. About the seventh day after a culture is started a small percentage of the individuals become associated in pairs, superficially resembling conjugants. Upon closer examination, however, no protoplasmic fusion or even contact can be detected. Instead the long cilia of the anterior end of one individual appear to be stuck to the anterior portions of the ridge of the other, thereby holding the two organisms together. This association is rather loose and the two separate soon after they are placed on a slide. We do not know whether or not this reaction has any significance in the life history of the ciliate, but we are inclined to think that it may represent simply an increased stickiness of the ridge-ectoplasm during the initial period of food storage.

We have evidence that indicates that the establishment of the normal reproductive rate of a freshly inoculated culture of *Perispira* is influenced by the time during which the food organisms had been growing in the medium before the inoculation of the ciliates. This is a conditioning effect and is being investigated for a future report. It is also

true that the physiological condition of the food organism determines, to a large extent, the ability of the *Perispira* to feed. Recently we have had cultures in which the *Euglena* (grown through many transplants in 1 per cent Difco Tryptone) were smaller than normal and possessed a reduced number of chloroplasts. Although these *Euglena* were active, the *Perispira* ingested few of them and as a consequence remained relatively small and reproduced very slowly. When these ciliates were presented with normal flagellates, however, they began to eat rapidly. In order to keep our food organisms in good condition, therefore, it seems necessary to maintain stocks in an acetate-containing medium, such as Medium D described by Hall (1937).

DISCUSSION

The establishment of *Perispira ovum* in bacteria-free culture along with *Euglena gracilis* as the food organism affords an opportunity for the study of a carnivore under controllable conditions. The fact that cultures remain alive for only a short time when in association with their natural bacterial flora, while sterile cultures last for months, emphasizes again the harmful effects of some types of bacteria. This situation was pointed out in a previous work (Kidder and Stuart, 1939a) on a bacteria-feeding species and seems to hold equally well for this carnivore.

An analysis of the reproductive rate and the rate of increase in cell size is interesting. The lag phase is not a period of inactivity. The ciliates are feeding rapidly and increasing in size at a higher rate than at any other time. This is also true of the majority of bacteria (Henrici, 1928), although the mechanism of size increase appears to be different (for a discussion of this point see Stephenson, 1939). A similar analysis of the size changes of *Leucophrys* would be interesting. No data on this point are given in Brown's (1940) paper. During the first part of the logarithmic phase the size measurements indicate that reproduction, while it certainly decreases cell size (by one half), does not keep pace with individual growth resulting from feeding. These ciliates may be said to eat faster than they divide. After the concentration of the food organism begins to decline there is no decrease in reproductive rate, so that, for the first time, reproduction overtakes and passes feeding rate. Experiments wherein food organisms would be continually added to the culture would be very interesting in this connection. Such experiments are planned.

Factors limiting the growth of carnivorous ciliates, grown as described above, have not been analyzed. Under the conditions of our

experiments perhaps the most important single factor is food, but there are many others. As compared to a culture of a purely saprozoic species these carnivores offer certain advantages and certain disadvantages for growth studies. One of the most complicated factors is an evaluation of the balance between the growing food organism and the carnivore. We will be nearer a solution of some of the problems when we formulate a medium which will not support multiplication of the food flagellates, but will maintain them in a trophic condition. So far none of the media used satisfies these requirements.

SUMMARY

1. *Perispira ovum* is a member of the family Spathidiidae. It is carnivorous, feeding on euglenoid flagellates.

2. A morphological description is given in order to establish the identity of the experimental organism.

3. A description of the mechanism of feeding is given. *Perispira* possesses a spiral ridge to which the flagella of euglenoid flagellates adhere. The flagellum and then the body of the prey is drawn into the mouth of the ciliate where it eventually rounds up and is digested. The cytoplasm of the prey is digested first, then the chloroplasts and lastly the paramylum bodies.

4. *Perispira* was rendered bacteria-free and established in culture with sterile *Euglena gracilis* as food.

5. When the population of such a culture is followed, a typical growth curve results. If ciliates from an old culture are used as the inoculum there follows a period of lag, a logarithmic growth phase, a phase of negative growth acceleration, a stationary phase and a phase of slow decline.

6. Cell size increases during the lag phase and early logarithmic phase but decreases rapidly during the late logarithmic phase and phase of negative growth acceleration. Cell size is correlated with the presence of food and the rate of cell division.

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